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COMPARISON OF PHYSICAL PROPERTIES OF THREE SILKS FROM *NEPHILA CLAVIPES* AND *ARANEUS GEMMOIDES*

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ABSTRACT. Orb-web weaving spiders synthesize and use a variety of silks, each having different properties suited to their particular functions. Three of these silks were collected from two different species of spiders and subjected to physical/mechanical testing. The major ampullate (dragline), minor ampullate, and cocoon silks of both *Nephila clavipes* and *Araneus gemmoides* were load tested on an Instron Universal test frame to compare their physical properties. The single fibers of major, minor, and cocoon silk of *Nephila* appear to be more elastic than that of *Araneus*. *Araneus* silks, on the other hand, appear to be stronger, requiring a higher stress to break the fiber than that of *Nephila*.

Through millions of years of evolution, spiders have been specializing their use of silks, to the point that many are completely dependent on the silks they produce for survival. The orb-web weaving spiders are a key example of this. They spin highly complex webs from several different types of silk, produce silk to protect their eggs, and silk to swathe their prey for storage. Orb-web spiders have at least six sets of silk-producing glands (Lucas 1964; Koover 1987), each synthesizing a different fiber for use in a specific application. Each fiber is composed almost entirely of protein.

METHODS

Preliminary data were gathered for densities and fiber diameters of various silks before the initial testing took place. The densities of six different silks: dragline, minor ampullate, and cocoon silk for both *Nephila clavipes* and *Araneus gemmoides*, were calculated using a chloroform/ethyl acetate gradient. These values along with the length and mass of each silk were used to determine the average diameter of each silk. The average diameter was also determined by use of a Bausch & Lomb microprojector, projecting the silk onto a digital measuring pad by which at least ten points along the length of a fiber were randomly chosen and measured.

Single fibers of dragline and minor ampullate silk of *Nephila clavipes* and *Araneus gemmoides* were hand-drawn at a rate of 10 cm/sec while the cocoon fibers were teased from the cocoons. Fibers were then fixed to lengths of black construction paper with plastic ("Scotch") tape and water-based white ("Elmer's") glue. The strips of black construction paper used for the dragline and minor ampullate silks contained a 4.0 cm diamond-shaped aperture in the center (Fig. 1). The strips used for the cocoon silks contained a 2.5 cm aperture in the center. Two pieces of clear plastic tape were placed on each end of the silk width-wise and a droplet of white glue was placed on the silk and paper between the plastic tape and the aperture. The glue drops were placed at the tips of the aperture. This provided for a consistent gauge length for each specimen constructed.

In addition to the single fibers, multi-fiber bundles of 100 silk fibers were forcibly drawn and collected (Xu & Lewis 1990) using a slotted cone-shaped spool. The multi-fiber bundle was removed by compressing the cone and the bundle was fastened to construction paper as described above. Multi-fibers were collected for major and minor ampullate silks for both species.

After the glue was allowed to dry, the ends of each sample were clamped into the grips of an Instron Universal test frame (model 1125) and the sides of the aperture were cut, allowing the 4.0/2.5 cm length of spider silk to be suspended between the heads. The grips were separated enough to remove any slack in the fiber, but not enough to induce a tensile load. The grips were then allowed to separate at a constant rate of 5 mm/min.

The force exerted as the heads separated was recorded by computer for the duration of the experiment. The data points were collected at a sampling rate of 2.0 or 0.6 per second, depending on the computer used. The separation distance between the heads was also recorded by computer. The strain (percentage elongation beyond the original fiber length) was determined from

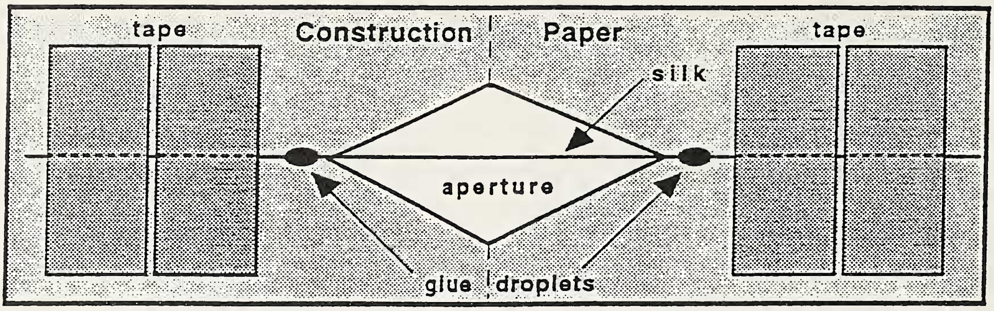
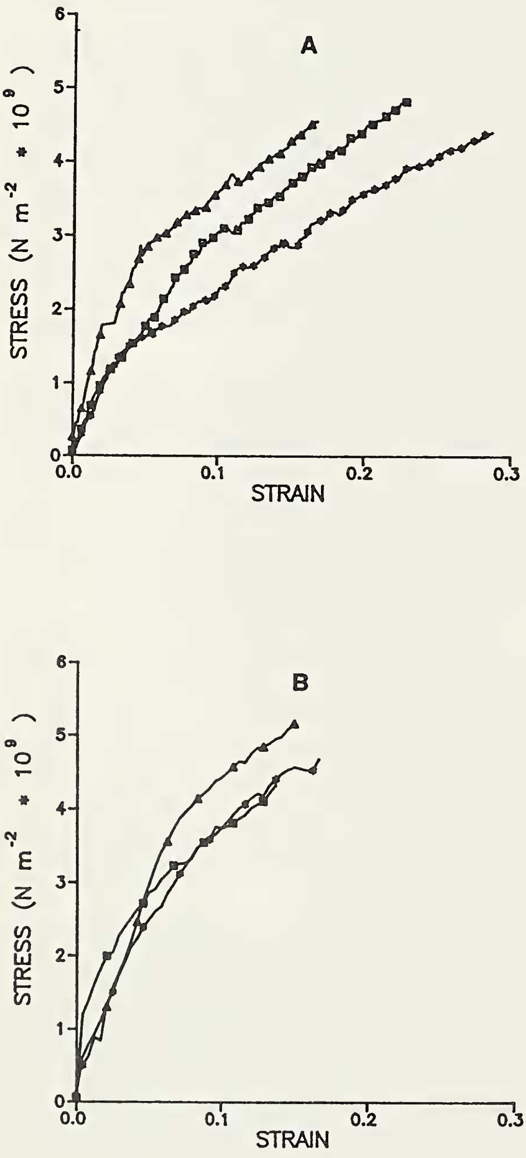


Figure 1.—Construction paper template. Using clear tape and white ("Elmer's") glue, silk was fastened to template as shown here and described in methods.



this measurement. The stress (force per unit cross-sectional area) could also be calculated since the diameter of the fiber was previously determined. These data were organized using Lotus 123 (Lotus Development Corporation, 1985) which could then be transformed into stress/strain graphs using Grapher Version 1.77 (Golden Software, Inc., 1988).

A series of tests was conducted to measure the compliance of the whole system including the Instron, paper, clamps, and load cell. The system compliance was experimently determined per the procedure described in ASTM (Standard Test Method D-3379-75, 1986). Four different gauge lengths (apertures), of 2.5, 4.0, 6.0, 8.0 cm in length, were tested using dragline silk of *Nephila clavipes* in a multi-fiber bundle of 100 fibers.

Sixteen multi-fiber bundles were collected for each gauge length and fixed to a black construction paper template in the same manner as mentioned above. By performing identical tests on sets of fibers that only varied in gauge length, the system compliance was experimently determined. To obtain the true compliance of each silk specimen tested, the compliance of the test system needs to be subtracted from the compliance indicated by the individual test specimen data. The true compliance is then used to cal-

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Figure 2.—Single fibers of major ampullate silk. (A) represents three separate samples of *Nephila clavipes* while (B) represents three separate samples of *Araneus gemmoides*. Both were tested for mechanical resistance as explained. The Instron cross-head speed was 5 mm/min.

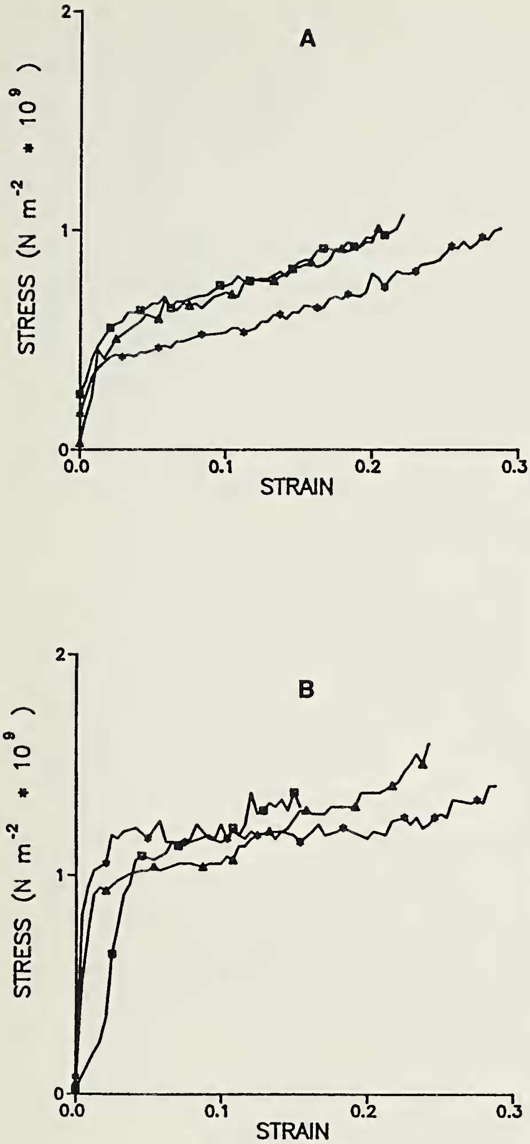

Figure 3.—Single fibers of minor ampullate silk. (A) represents three separate samples of *Nephila clavipes* while (B) represents three separate samples of *Araneus gemmoides*. Both were tested for mechanical resistance as explained. The Instron cross-head speed was 5 mm/min.

culate the silk specimen elongation, strain and Young's modulus.

To test for the effect of moisture on silk, two sets of samples of *Nephila clavipes* multi-fibers were prepared as previously described. The first set was loaded into the Instron with the silk tension adjusted to zero. It was then wetted with distilled water using a spray bottle. The tension induced in the silk fiber by the water was then relieved by slightly moving the specimen grips closer together. The grips were then allowed to separate at a constant rate. Load and elongation data were recorded throughout this procedure. The second set was also loaded into the Instron with the silk tension adjusted to zero and wetted with distilled water, but was not re-zeroed before allowing the crossheads to separate.

Finally, a series of tests was conducted to test if the rate of crosshead separation would affect the results of the data collected on the Instron. Since most of our work was completed using a crosshead speed separation of 5 mm/min, we decided to test the effect of a higher rate of separation to be certain that the rate was not influencing the data we collected. Samples were run at 5, 20, and 100 mm/min crosshead speed separation using single fibers of major and minor ampullate silk from *Nephila clavipes* as described above.

RESULTS

The silk densities determined by the chloroform/ethyl acetate gradient were recorded as follows: The silk densities of *Nephila clavipes* for major ampullate, minor ampullate, and cocoon silk were 1.36, 1.19, 1.34 respectively. The silk densities of *Araneus gemmoides* for major ampullate, minor ampullate, and cocoon silk were 1.27, 1.18, and 1.26 respectively.

Using these densities along with the mass and length of the various silks and with the aid of a microprojector, the estimated diameters of each silk were determined as follows: The average minimum silk diameters of *Nephila clavipes* for major, minor, and cocoon silk were estimated to be 3.00, 2.50, and 7.00μ respectively. The average minimum silk diameters of *Araneus gemmoides* for major, minor, and cocoon silk were estimated to be 2.50, 2.00, and 6.00μ respectively.

Using these diameters, the area of a silk fiber was calculated and these areas, in turn, were used to calculate the stress necessary to break a particular fiber. Since 100 single fibers make a multi-fiber bundle, the areas of the multi-fiber samples for major and minor ampullate silk of both spider species were simply 100 times that of the single fiber areas calculated for each.

Figures 2–6 show the stress/strain graphs recorded for each of the silks tested. Each graph contains three typical silk representives of that particular silk. The *Nephila* data and the *Araneus* data both show a biphasic response typical of many fibers.

Figure 2A represents a single fiber of major ampullate silk from *Nephila clavipes*. Figure 2B represents a single fiber of major ampullate silk from *Araneus gemmoides*. The change to a different stress/strain relationship occurs at about a 4–5% elongation of the fiber for both species of spider. For *Nephila*, the final breaking point for this fiber occurs at about a 23 ± 5% (n = 10) extension with a final stress of 4.6 ± 0.2 × 10^9 Nm^{-2}. For *Araneus*, the final breaking point for this fiber occurs at about a 15 ± 2% (n = 10) extension with a final stress of about 4.7 ± 0.5 × 10^9 Nm^{-2}.

Figure 3A represents a single fiber of minor ampullate silk from *Nephila clavipes*. Figure 3B represents a single fiber of minor ampullate silk from *Araneus gemmoides*. The change to a different stress/strain relationship occurs at about a 2–3% elongation of the fiber for both species of spider. For *Nephila*, the final breaking point for this fiber occurs at about a 25 ± 3% (n = 10) extension with a final stress of 0.96 ± 0.05 × 10^9 Nm^{-2}. For *Araneus*, the final breaking point for this fiber occurs at a 22 ± 7% (n = 10) extension with a final stress averaging about 1.4 ± 0.1 × 10^9 Nm^{-2}. The breaking stress of the minor ampullate silk of *Araneus* is notably higher than that of *Nephila*.

Figure 4A represents a single fiber of cocoon silk from *Nephila clavipes*. Figure 4B represents a single fiber of cocoon silk from *Araneus gemmoides*. The change to a different stress/strain relationship occurs at about a 4–5% elongation of the fiber for both species of spider. For *Nephila*, the final breaking point for this fiber occurs at about a 24 ± 2% (n = 10) extension with a final stress of 1.3 ± 0.2 × 10^9 Nm^{-2}. For *Araneus*, the final breaking point for this fiber occurs at about a 19 ± 2% (n = 10) extension with a final stress of 2.3 ± 0.2 × 10^9 Nm^{-2}. As in the minor silk, The cocoon silk of *Araneus* appears to be stronger than that of *Nephila*.

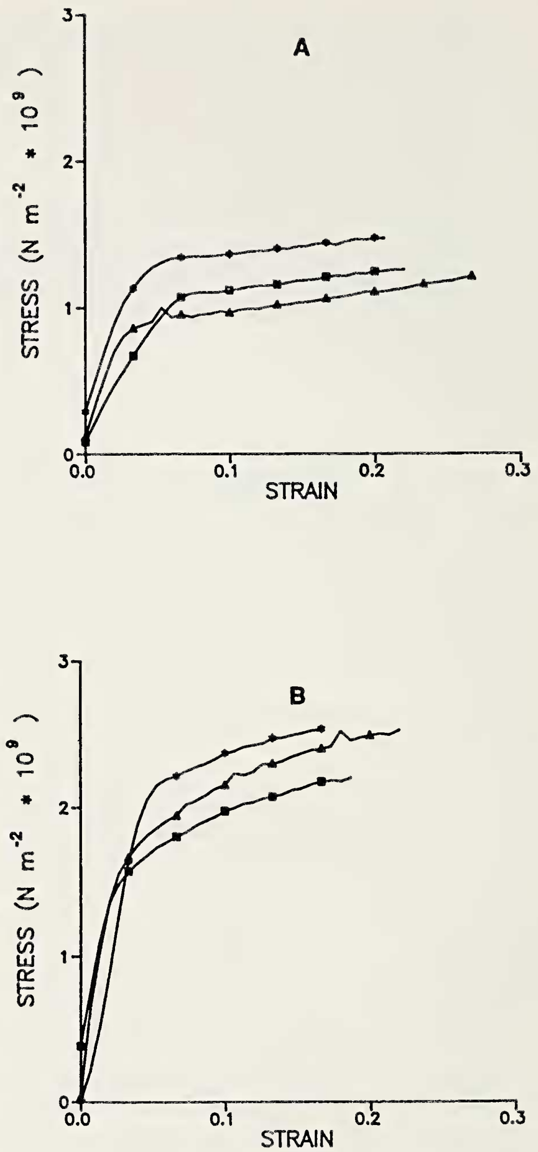

Figure 4.—Single fibers of cocoon silk. (A) represents three separate samples of *Nephila clavipes* while (B) represents three separate samples of *Araneus gemmoides*. Both were tested for mechanical resistance as explained. The Instron cross-head speed was 5 mm/min.

Figure 5A represents a multi-fiber bundle of major ampullate silk from *Nephila clavipes*. Figure 5B represents a multi-fiber bundle of major ampullate silk from *Araneus gemmoides*. The change to a different stress/strain relationship occurs at about a 2–3% elongation of the fibers for *Nephila*, and a 4–5% elongation for *Araneus*. For *Nephila*, the final breaking point for these fibers occurs at a 32 ± 8% (n = 10) extension with a final stress of 2.8 ± 0.2 × 10^9 Nm^{-2}. For *Araneus*, the final breaking point for these fibers occurs at a 64 ± 6% (n = 10) extension with a final stress of 1.9 ± 0.1 × 10^9 Nm^{-2}.

Figure 6A represents a multi-fiber bundle of minor ampullate silk from *Nephila clavipes*. Fig-

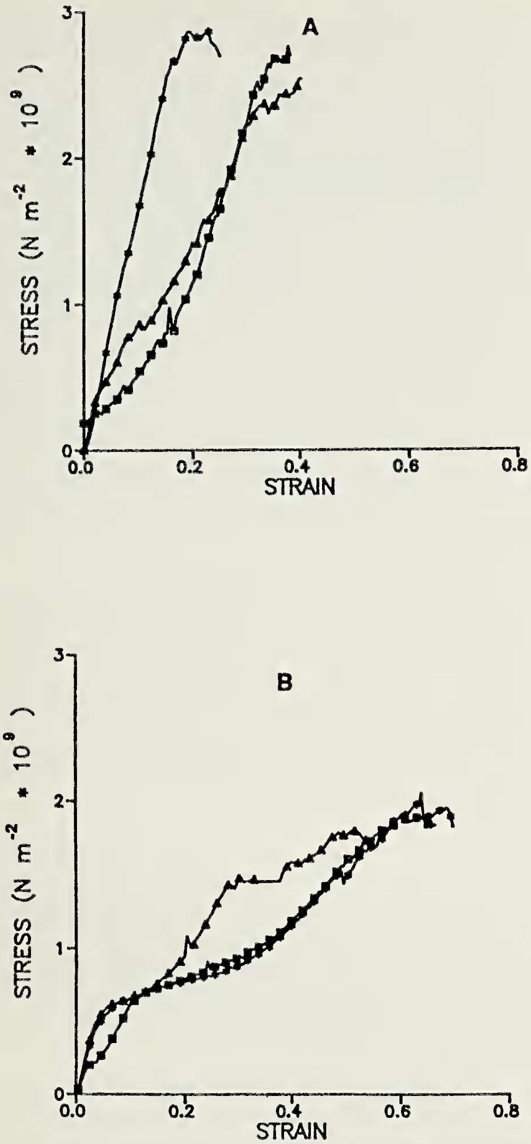

Figure 5.—Bundles of 100 fibers of major ampullate silk. (A) represents three separate samples of *Nephila clavipes* while (B) represents three separate samples of *Araneus gemmoides*. Both were tested for mechanical resistance as explained. The Instron cross-head speed was 5 mm/min.

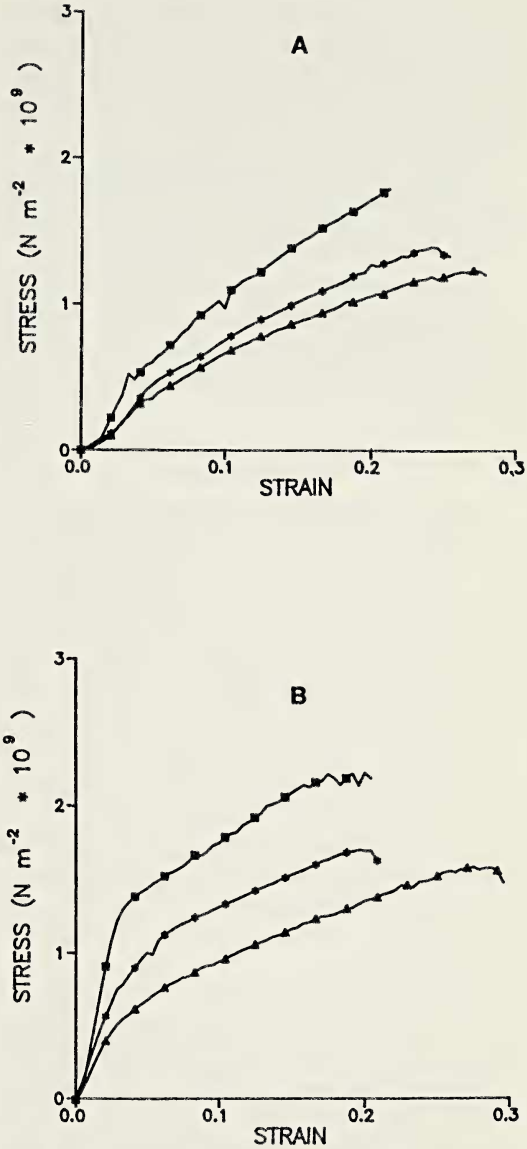

Figure 6.—Bundles of 100 fibers of minor ampullate silk. (A) represents three separate samples of *Nephila clavipes* while (B) represents three separate samples of *Araneus gemmoides*. Both were tested for mechanical resistance as explained. The Instron cross-head speed was 5 mm/min.

ure 6B represents a multi-fiber bundle of minor ampullate silk from *Araneus gemmoides*. The change to different stress/strain relationship occurs at a 4–5% elongation of the fibers for *Nephila*, and a 2–3% elongation for *Araneus*. For *Nephila*, the final breaking point for these fibers occurs at a 25 ± 3% (n = 10) extension with a final stress of $1.6 \pm 0.4 \times 10^9$ Nm^{-2}. For *Araneus*, the final breaking point for these fibers also occurs at a 26 ± 4% (n = 10) extension, but with a higher final stress of $1.9 \pm 0.3 \times 10^9$ Nm^{-2}.

The system compliance, experimently determined, for the Instron set-up that was used for each specimen tested, was found to be extremely small with respect to the compliance indicated for each specimen tested. Thus, it was not necessary to adjust the data with respect to the system compliance since this value was insignificant.

The effect of moisture on the silk proved to be small overall, with a change in elongation of the fiber of about 1%. The stress necessary to break the fiber was the same for both tests.

The testing of the different rates of crosshead speed separation of 5, 20, and 100 mm/min showed no significant differences between the three with respect to the stress required to break the fibers.

DISCUSSION

Although substantial work has been done on the mechanical properties of spider silk (Gosline et al. 1984), there were several areas that had not been well studied. The first was the uniformity of the fiber diameter which clearly would affect the calculated tensile strengths. Using the optical measuring system, the fibers were found to vary by more than a factor of two in the maximum and minimum diameters. In order to present an accurate value for tensile strength, we assumed that the fiber was most likely to break at the minimum diameter. We thus measured the ten smallest diameter points in several sections of the different silks within the 4.0/2.5 cm template. The average of these points was used for our calculations.

The data presented here for tensile strengths of single fibers is higher than other published values (Work 1976) due to the smaller diameter used for the calculations. However, since the calculations were done in a similar manner for all silks, the comparison data is consistent. For both *Araneus* and *Nephila*, the three silks show an identical ordering of strength. The major ampullate silk is substantially stronger than either of the other two silks. The cocoon silk is next, followed closely by minor ampullate silk. In comparing the two species, the *Araneus* silks are stronger than the corresponding silks from *Nephila*. The values are over 50% greater for the cocoon and minor ampullate silks, but less than 5% for major ampullate silk. The multiple fiber bundles showed a wide range over which the fibers broke which is not surprising in view of the range of diameters observed. Thus, it is not possible to determine a useful value for the tensile strength of the fiber bundles.

The elongation values found for the major ampullate silk from both species are consistent with values found by others and was reversible as well (Work 1976). Minor ampullate silk showed a larger elongation (over 20%) than previously found and this elongation was not reversible. To our knowledge, cocoon silk has not previously been studied and showed a similar elongation to minor ampullate silk. An interesting and previously undescribed feature of *Nephila* cocoon silk is its brittle character. It was extremely difficult to obtain any 4.0 cm samples without the fibers breaking while being put on the paper template so we had to settle for 2.5 cm samples instead. The elongation data for the multi-fiber bundles was complicated by physical interactions between the fibers after they broke. This greatly increased the elongation over that of the single fibers.

This comparison of the three silks from two different species indicates that the different fibers each possess unique characteristics. The fibers show a remarkable similarity between the two species indicating that evolutionary pressure has maintained the fiber characteristics. This is probably due to an identical usage of the different fibers in the two species.

CONCLUSIONS

Single fibers of *Nephila clavipes* major, minor, and cocoon silk are more elastic than those of *Araneus gemmoides*, respectively. Overall, single fibers of *Araneus* major, minor, and cocoon silk appear to be stronger than that of *Nephila*, respectively.

Comparing the silks within each species, a single fiber of *Nephila* major ampullate silk appears to be quite a bit stronger than that of minor or cocoon silk, requiring an average stress of $4.6 \pm 0.2 \times 10^9$ Nm^{-2} to break it. The cocoon silk would be the next strongest requiring an average stress of $1.3 \pm 0.2 \times 10^9$ Nm^{-2} to break its fiber. Finally, the minor ampullate silk which appears to be the weakest of the three, requires an average stress of $0.96 \pm 0.05 \times 10^9$ Nm^{-2} to break it.

A single fiber of *Araneus* major ampullate silk also appears to be stronger than that of its minor or cocoon silks requiring an average stress of $4.7 \pm 0.5 \times 10^9$ Nm^{-2} to break it. The cocoon silk appears to be the next strongest needing an average stress of $2.3 \pm 0.2 \times 10^9$ Nm^{-2} to break its fiber. Finally, just like the *Nephila* minor silk, the *Araneus* minor ampullate silk appears to be the weakest of the three requiring an average stress of $1.4 \pm 0.1 \times 10^9$ Nm^{-2} to break it.

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